EXHIBIT 45

PUBLIC HEALTH 165 (2018) 48–57

Available online at www.sciencedirect.com

Public Health

journal homepage: www.elsevier.com/puhe

Original Research

Evaluating biological plausibility in supporting evidence for action through systematic reviews in public health

J. Dailey [a], L. Rosman [b], E.K. Silbergeld [c,*]

[a] Johns Hopkins University, Whiting School of Engineering, Department of Materials Science, USA
[b] Johns Hopkins University, Johns Hopkins School of Medicine, Welch Medical Library, USA
[c] Johns Hopkins University, Bloomberg School of Public Health, Department of Environmental Health and Engineering, USA

ARTICLE INFO

Article history:
Received 20 April 2018
Received in revised form
28 August 2018
Accepted 31 August 2018
Available online 25 October 2018

Keywords:
Biological plausibility
Systematic reviews
Mechanisms
Antimicrobial resistance
Food borne infections

ABSTRACT

Objectives: The objective of this research was to develop and test methods for accessing and evaluating information on the biological plausibility of observed associations between exposures or interventions and outcomes to generate scientific evidence for action consistent with practice in systematic reviews.

Study design: To undertake this research, we used the example of the observed associations between antimicrobial use in food animals and increased risks of human exposures to antimicrobial resistant pathogens of zoonotic origin.

Methods: We conducted a scoping search using terms related to biological plausibility or mechanism to identify key references. As recommended by these references, we also used expert consultation with researchers and a public health informationist. We used their recommendations, which included expert consultation, to identify mechanisms relevant to biological plausibility of the association we selected to test. We used the reviews conducted by the World Health Organization (WHO) Guidelines Development Group in support of reducing antimicrobial use in food animal production to populate our model for assessing biological plausibility.

Results: We were able to develop a transparent model for biological plausibility based on the adverse outcome pathway used in toxicology and ecology. We were also able to populate this model using the WHO reviews.

Conclusions: This analysis of biological plausibility used transparent and validated methods to assess the evidence used in systematic reviews based on the observational studies accessed through searches of the scientific literature. Given the importance of this topic in systematic reviews and evidence based decision making, further research is needed to define and test the methodological approaches to access and properly evaluate informa tion from the scientific literature.

© 2018 The Royal Society for Public Health. Published by Elsevier Ltd. All rights reserved.

* *Corresponding author.*
E mail addresses: jdailey4@jhu.edu (J. Dailey), Lrosman1@jhmi.edu (L. Rosman), esilber2@jhu.edu (E.K. Silbergeld).
https://doi.org/10.1016/j.puhe.2018.08.015
0033 3506/© 2018 The Royal Society for Public Health. Published by Elsevier Ltd. All rights reserved.

Introduction

Evidence based methods in medicine and other health related fields have emphasized biological plausibility as an important element in assessing the strength of evidence since the work of Bradford Hill.[1,2] As noted in a recent review of cancer risks, information on biological plausibility is particularly important as a complement to associations observed in epidemiological studies.[3] For observational studies, the quality of evidence is often judged weaker than the evidence based on randomized controlled studies. These study designs, which are necessary, given the ethical rami fications of interventions in public health, are considered to be less able to eliminate the effects of residual bias. As a consequence, evaluating biological plausibility or mecha nisms may be of particular value in assessing the strength of evidence from this literature. This has been recognized by several regulatory agencies, including the US Environmental Protection Agency and the European Food Safety Agency, as well as by the WHO and CODEX.[4,5]

However, despite the importance of the topic, there are no generally accepted methods for evaluating biological plausi bility, and many reviews discussing these mechanisms include only general statements on relatively non specific physiolog ical events or target organs with no supporting references.

Our research question concerned the biological plausibility of observed associations between antimicrobial (AM) use in agriculture and increased risks of human exposures to drug resistant zoonotic pathogens. There are many reviews of this topic, including two recent systematic reviews. One of these systematic reviews was undertaken by the WHO Guidelines Development Group to support its task to develop evidence based recommendations and guidelines to reduce antimicro bial resistance related to agricultural use.[5] An additional sys tematic review was published independently.[6] The WHO systematic review used the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) method ology to assess the quality of the evidence, and following the GRADE criteria, the evidence was rated of low confidence.[7] The other systematic review[6] used a modified GRADE approach for evaluating evidence in which the 'sufficient component' causal model proposed by Rothman was incorporated.[8]

Assessments using GRADE can cause confusion among users of guidance based on these reviews. A statement issued by the United States Department of Agriculture (USDA) shortly after the publication of the WHO guideline referred to this 'low quality evidence' as effectively disqualifying any WHO rec ommendations, despite the surrounding analyses and expert opinion.[9] To provide additional support for this evidence, we undertook an assessment of the biological plausibility of the observed associations between antimicrobial use in food ani mal production and increased risks of human exposures to and infections by antimicrobial resistant zoonotic pathogens.[10]

Methods

We used scoping reviews and expert consultation to identify two articles with general discussions of methods related to biological plausibility.[11,12] From these articles , we identified the following search terms 'methods'[Subheading] OR 'methods'[All Fields] OR 'methods'[MeSH Terms]) AND ('research design'[MeSH Terms] OR ('research'[All Fields] AND 'design'[All Fields]) OR 'research design'[All Fields] OR 'tes t'[All Fields]) AND ('biology'[MeSH Terms] OR 'biology'[All Fields] OR 'biological'[All Fields]) AND 'plausibility'[All Fields] to access articles from the biomedical literature with more detailed methods for defining causal pathways in terms of molecular and genetic mechanisms.[3,13,14] With further expert consultation, we further accessed articles from the toxicology and ecology literature that defined mechanisms as causal pathways in the context of adverse outcome analytic meth ods.[15–17] We used the adverse outcome pathway model as it more closely represents the research question we sought to investigate, that is, a series of discrete mechanistic events not as strictly limited to one molecular pathway as in Lewis et al.[3] This methodology uses schematics to represent pathways, as shown in an example in Fig. 1.

To apply this model, we used a scoping review approach, including reviews, to identify sources of information on the biological plausibility of observed associations between antimi crobial use in agriculture and increased risks of human exposure to and infection by antimicrobial resistant pathogens from food animals. We developed and populated a similar structure for this review based on a conceptual structure that represents a sequence of mechanisms involved in the emergence and dissemination of antimicrobial resistance.[18–21] To this model, we added the routes that connect these events in agriculture to human exposure. Consistent with the WHO practice in guideline development, we sought a global sampling of articles.

Our conceptual model is shown in the following section (Fig. 2) (see Figs. 3 and 4).

In this model, antimicrobial pressure includes the following variables: volume of antimicrobial use, concentra tions of antimicrobials encountered by pathogens in animal guts, duration of antimicrobial use, and use of >1 antimicro bial at a time. Selection for resistance includes both natural selection through evolutionary mechanisms and horizontal gene transfer (HGT) of one or multiple resistance genes. Resistance dissemination includes clonal expansion of resis tant organisms and gene flow among organisms through HGT involving mobile genetic elements (MGEs), conjugation, and other mechanisms. Reservoirs include the resistome (defined as microbial resources of resistance genes) and the mobilome (defined as microbial resources for enabling intercellular transfers of resistance genes) that are available within microbiomes in hosts and the external environment.[22] We defined human exposure pathways to include direct and in direct animal:human contact; releases from animal confine ment houses; waste disposal; and consumption of food products derived from animals.[23,24]

Results

STEP 1 Antimicrobial pressure → selection for resistance

Fundamental to our understanding of mechanisms involved in the emergence of antimicrobial resistance is the fact that

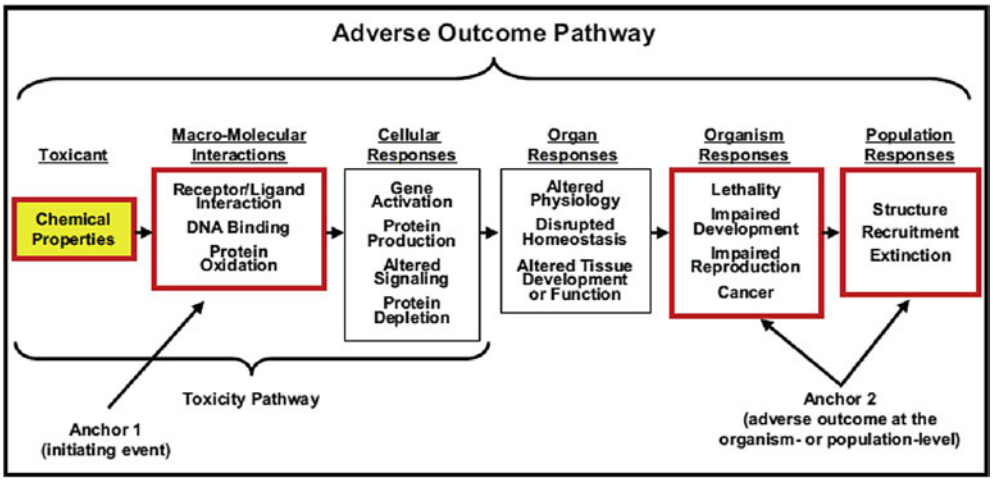

Fig. 1 – An adverse outcome pathway as used in toxicology to define events in a causal sequence connecting exposures to outcomes at the population level.[15]

antimicrobial resistance is inherent within microbial pop ulations. For billions of years, microbes have produced almost all currently used antimicrobial molecules in response to intensive competition for resources and survival within the microbiome.[25] In this context, antimicrobial resistance (AMR) evolved as an evolutionary mechanism by which microbes survived through natural selection by random gene mutation that encoded traits that conferred resistance to these natural biotoxins.

In contrast, human uses of antimicrobials are very recent, beginning in the early 1940s. Yet due to this prehistory, resistance mechanisms were already present within bacterial populations.[26] During the first years of experimentation by Fleming and others, resistance was recognized as a conse quence of exposure. Evolutionary theory explained the emergence of antimicrobial resistance as a process of random genetic mutations that conferred biological resistance to drugs.[27] This theory also supported the assumption that each instance of resistance required either vertical transmission from the replication of a resistant organism or a separate evolutionary event. At first, little was known of the specific mutations or molecular mechanisms of AMR, but with the rapid development of molecular genetics, these altered pro teins were identified.[28]

Evolutionary theory also supported the assumption that there was a cost of resistance involving a trade off between resistance and the growth rate (the *r*K selection theory). Without this cost, bacteria would be equally likely to be resis tant or susceptible in the absence of AM pressure, and with the removal of AM pressure, the prevalence of resistant strains would decrease. However, experimental observations contra dicted theory, which was amended to include more complex evolutionary responses, such as 'bet hedging,' by which mi crobial populations under AM pressure could acquire addi tional mutations to compensate for the cost of resistance.[29]

Over the past 50 years, a substantial revolution has occurred in our understanding of the mechanisms by which AMR emerges and is disseminated. The current research now sup ports the hypothesis that HGT, rather than mutation, is the major mode by which bacteria (and other microbes) respond to antimicrobial pressure.[30] Horizontal or lateral gene transfer among live cells was observed, although not understood mechanistically, as early as 1928.[31] Bacteria use several mech anisms to share resistance genes, including conjugation or exchange through direct cell:cell contact, transformation or incorporation of naked DNA from disrupted organisms in the extracellular environment, and transduction involving transfer of genetic material by transposable genetic elements.[27,32] Later experiments demonstrated mechanisms by which donor cells initiate plasmid mediated gene transfer and how antimicro bials stimulate intercellular signaling between susceptible and resistant bacterial strains to initiate events including gene transcription that facilitate HGT from chromosomal DNA within the donor cell and responses such as swarming within the susceptible recipient organisms.[32–34] The mechanisms by which resistance genes that are transferred among cells can be incorporated into the chromosomal genome of the recipient cell and expressed are also understood.[35]

Fig. 2 – A conceptual model of the mechanisms by which use of antimicrobials in food animal production increases the risks of antimicrobial resistance and exposure of human populations to pathogenic bacteria.

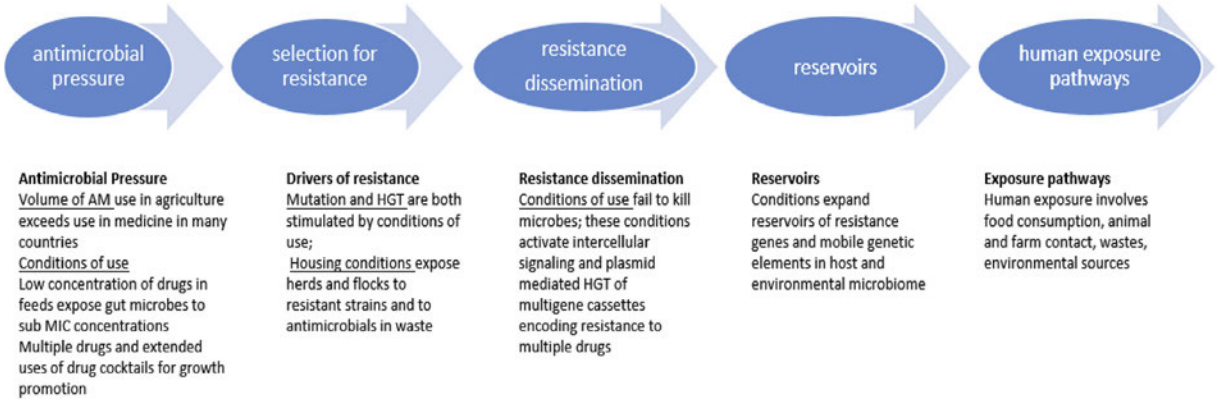

Fig. 3 – Conceptual model with an explanatory text to describe the biological plausibility between agricultural AM use and risk to human population. MIC, minimum inhibitory concentration; HGT, horizontal gene transfer; AM, antimicrobial.

Concentrations of antimicrobials

The conditions of AM use also affect resistance emergence and dissemination. The most significant overall risk factor driving AMR emergence in any setting is the volume of drug use. Associations between overall drug use and prevalence of AMR have been shown by cross sectional comparisons of national drug use data[36] and longitudinally after bans on the use of certain drugs in agriculture.[37] In addition, the concentrations of AMs to which microbes are exposed are also significant. Exposures to subtherapeutic concentrations of AMs (defined by bioassay at concentrations below the minimum inhibitory concentration [MIC]) are particularly effective as drivers of selection for AMR. This seemingly paradoxical observation reflects the Nietzschean aspects of bacteria that which does not kill them makes them strong. Higher concentrations of AMs (greater than or equal to the MIC) kill bacteria, whereas sublethal exposures stress but spare bacteria. As a consequence, these stressful but non lethal conditions are particularly effective as drivers of selection for AMR through two mechanisms: increased growth and mutation rates and enhanced transfer of resistance plasmids and conjugative transposons.[38] The survivors acquire resistance through these mechanisms and increased incorporation of resistance genes into chromosomal DNA. Continuous or prolonged low level AM use also expands the resistome and enhances the role of MGEs, including plasmids, in mediating the dissemination of resistance within the hosts and the environment within the microbiome.[22,39]

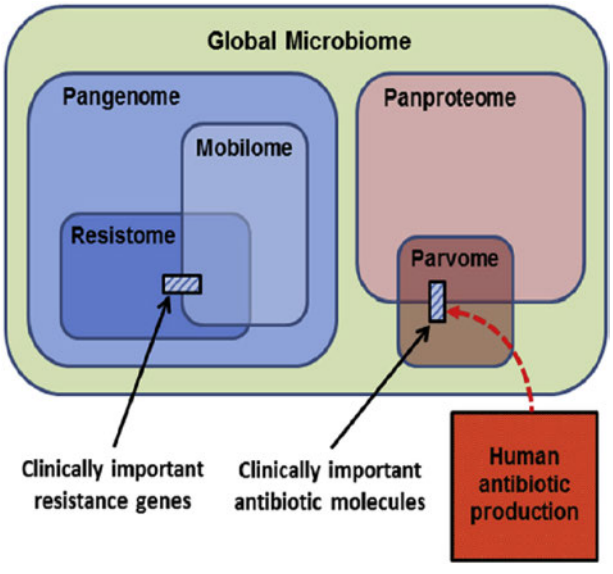

Fig. 4 – The relationships within the global microbiome and its pangenome including the resistome and the mobilome that support horizontal gene transfer in response to antimicrobial pressure including those genes encoding resistance to clinically important antimicrobials. The panproteome includes the gene products of the microbiome, including the parvome which includes clinically important antimicrobial molecules produced by humans.[22]

Use of multiple drugs

Repeated exposure to multiple AMs affects the emergence and dissemination of multidrug resistance through HGT of MGEs containing multiple resistance genes encoding resistance to several drugs. This results in both cross resistance and coselection. These mechanisms were first demonstrated in 1989, with experiments showing that cross resistance among antimicrobials can be selected by one drug represented in the multidrug resistant cassette.[40] Through HGT, bacteria not only exchange individual resistance genes but also cassettes of multiple resistance genes, which encode for coresistance to multiple antimicrobials. In other words, both pathogenic and non pathogenic bacteria can easily share an entire cookbook of avoidance tactics rather than a single recipe. In response to repeated exposures to multiple AMs, bacteria acquire 'genetic capital' in the form of sequential acquisition of resistance genes that can be transferred as a package through transposons within the mobilome.[41] These cassettes may be highly complex. *Salmonella* strain resistant to 13 antimicrobials was isolated from a child living on a farm who presented with ceftriaxone resistance; all but one of the genes encoding

multidrug resistance was on the same plasmid.[42] These multigene cassettes can include metal resistance genes such that coselection and cross resistance can also be driven by metals such as copper, cadmium, nickel, mercury, arsenic, and zinc.[43,44]

These conditions—use of concentrations of antimicrobials that result in subtherapeutic microbial exposures and use of multiple drugs in feeds—are common in the use of antimicrobials in poultry and livestock production. Another agricultural use is the long duration of repeated exposures for so called prophylaxis or metaphylaxis (preventive treatment in the expectation of but absence of diagnosed disease). This may also involve sublethal concentrations of antimicrobials.[45] These low dose and extended exposures to single or multiple antimicrobials condition networks of gene flow within the microbiome such that HGT is facilitated and the role of MGEs in mediating resistance gene flow is enhanced within the gut microbiomes in animal hosts and in the environment.[46]

STEP 2 Selection → Dissemination of resistance

HGT enables the rapid and efficient dissemination of resistance among bacteria (and other microbes) through highly efficient community signaling within the microbiome. This is in contrast to evolutionary mechanisms dependent on random mutation or clonal expansion. At low concentrations, horizontal transfers of resistance genes among microbes rather than vertical transmission or *de novo* mutations are now recognized as the most important mechanism and explanation for the rapid and far ranging dissemination of resistance within and among microbial populations within hosts and the environment.[47] These mechanisms support highly efficient mobilization of community resources of resistance. As a consequence, these resources are available to microbial networks that can be geographically distant and phylogenetically distinct.

Within and among microbial communities, HGT moves individual resistance genes and cassettes of multiple genes that encode for coresistance and coselection of resistance.[22,30] These mechanisms underlie the complexities and underscore the facility with which bacteria respond to antimicrobial pressure with both emergence and dissemination. Once a new resistance trait and gene emerges, it spreads rapidly among microbial communities. This dissemination is further facilitated by movement of bacteria through air and water, changes in methods of food animal production, and human behavior including food consumption patterns, global travel, and international trade in animals and food.

These mechanisms of dissemination are exemplified by the rapidity and global range of resistance of β lactams as evidenced in the emergence of extended β lactamases in response to the introduction of new cephalosporins.[48,49] Since the isolation of the first of these drugs in 1948, there are now five generations of cephalosporins. Bacteria have rapidly responded to each generation of new cephalosporins with increasing numbers of distinct β lactamase genes, now exceeding 1000.[48] Both resistant bacteria and resistance genes encoding extended spectrum β lactamase (ESBL) have spread rapidly and globally.[50] Moreover, ESBL resistance genes are frequently bundled with other resistance determinants in transposable gene cassettes.[51] Coselection has been suggested as the mechanisms for the rapidity of selection for resistance to novel cephalosporins such as carbapenem and colistin.[52]

STEP 3 Dissemination → Reservoirs of resistance

Resistance reservoirs include the resistome (defined as the biological resources for responding to antimicrobial pressure) and the mobilome (defined as all the biological resources for transferring genes in response to pressure).[22]

These reservoirs exist within microbes and as naked DNA within physiological niches such as the gut and ecological niches in the external environment. The increasing use of antimicrobials has enlarged the resistome and increased the activity of the mobilome.[22,53] Increases in antimicrobial resistance genes and class 1 integrons have been reported in animals fed antimicrobials and have been documented in studies of soils treated with animal wastes or veterinary antimicrobials.[47,54,55]

The environmental reservoirs of resistance may constitute the largest resources of these functions and are of specific concern in the context of agricultural uses through the release of untreated animal wastes containing resistance genes and antimicrobials that augment selection pressures within environmental microbiomes.[39]

The environmental resistome has been a source of resistance in pathogenic bacteria isolated from humans.[25] Because agriculture is situated directly within the physical and biotic environment, with numerous porosities from farm to fork, gene flow within and from food animal production contributes significantly to the environmental resistome.[56] This involves both the release of antimicrobials and resistance genes. Several studies have reported concentrations of antimicrobials in sediments impacted by aquaculture which are many fold greater than the minimal inhibitory concentrations for many drugs and pathogens.[57] In addition, multiple MGEs have also been measured in soils and sediments.[54] Empirical assessments of gene flow from agriculture into environmental microbiomes in soils and sediments have been published.[58]

STEP 4 Reservoirs → Exposure pathways

To evaluate the last step in this conceptual sequence, exposure of human populations to drug resistant pathogens from food animal production, we considered the role of the mechanisms discussed previously within the conditions and context of food animal production. Many of the conditions in food animal production resemble those risk factors that are conducive to the mechanisms of AMR emergence and dissemination first identified in healthcare settings, and for which interventions and guidance programs have been developed and implemented in many countries.[59] They are exacerbated by animal stress and crowding during growth stages and transport.[60,61]

In Fig. 2, we summarize the evidence for the role of mechanisms listed in Fig. 1 within the context of antimicrobial use in food animal production. We also indicate evidence supporting routes of exposure to these zoonotic pathogens from food animal production to human populations.

The food supply is the most significant pathway for human exposure to AMR pathogens from agriculture in terms of numbers of persons exposed, followed by multiple pathways of release to the environment. These two pathways operate both separately and in combination. In addition to con sumption of food products from animals, there is an under appreciated and overlooked pathway of food borne dissemination from the environment to crops consumed by humans. This is of particular risk when crops are grown with animal wastes (as in organic production) or with irrigation by surface water sources contaminated by run off from land disposal of animal[62,63].

The food and environmental pathways of exposure blur distinctions between health care and agriculture. Common sources of food are eaten inside and outside of healthcare facilities, and hospitals are located in environments where ambient air and water may be contaminated by agricultural releases. Moreover, people—patients, visitors, and healthcare personnel—move in and out of healthcare settings.[64] For this reason, there are no real barriers between the presence of AMR in agriculture and the entrance of these same AMR pathogens into healthcare settings. These factors make it impossible to identify sources of resistance or to allocate burdens of disease between clinical and agricultural uses. This circularity is shown in Fig. 5.

Regardless of the original source of AMR, in most cases, it is not possible to separate agricultural and clinical sources of genetic determinants of resistance in pathogens isolated from human populations, because genes and pathogens originating in agriculture quickly become sources of expo sures and infections in human communities and eventually move into healthcare settings, and strains in humans can be transferred to animal populations. This gene flow goes both ways. There is a well annotated history of the cross trans mission of so called 'livestock' strains of MRSA (ST398) from humans to animals and from animals to humans.[65] Some studies of ESBL+genes in *Escherichia coli* isolates from ani mals, including carbapenemase, suggest that this may represent contamination of the agricultural environment by human wastes.[66]

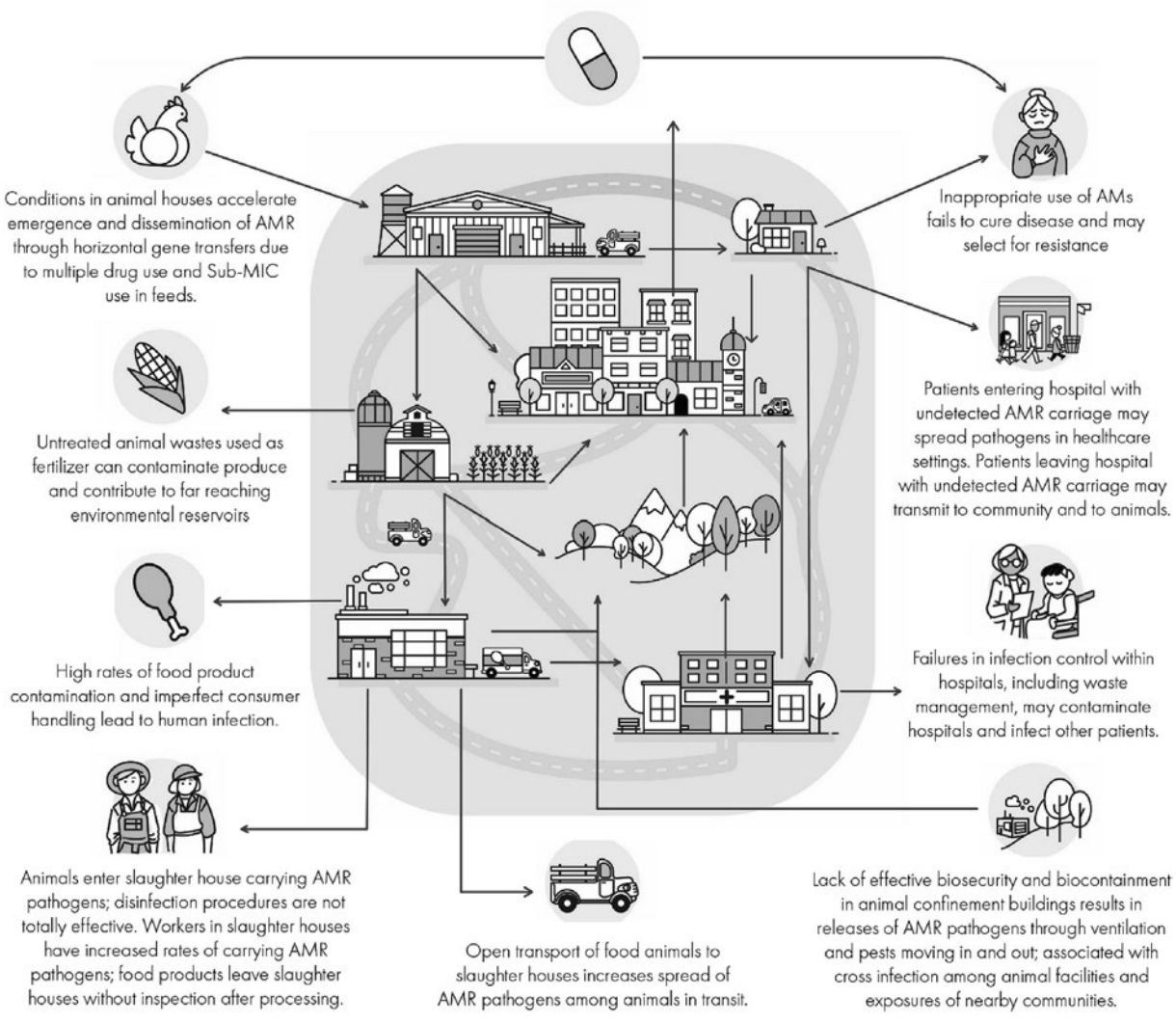

Fig. 5 – Illustration depicting complex relationships among and between multiple sources of AMR. AMR, antimicrobial resistance; MIC, minimum inhibitory concentration.

Discussion

We undertook this study to improve the evaluation of evi dence related to biological plausibility of associations observed in non RCT studies relevant to public health. The development of a transparent method for assessing the quality of these types of associations in observational studies is of high importance. The current assessment methods based on GRADE are not appropriate because of the inherent limi tations of public health studies. Moreover, the use of GRADE, as in the systematic reviews conducted by the WHO, may lead to underestimation of important findings. The USDA issued a statement shortly after the publication of the WHO guideline, which referred to this 'low quality evidence' as effectively disqualifying any WHO recommendations, despite the sur rounding analyses and expert opinion.[9] We selected the adverse outcome pathway approach based on our interest in the application of these methods for supporting the evidence derived from observational studies.

With expert consultation, we accessed articles describing general and detailed methods for organizing structural models representing biological plausibility through mecha nisms that link exposures to health outcomes. One of these methods uses a comprehensive information set based on the molecular biology of cancer (Lewis et al.),[3] and the other uses the more generalizable concept of adverse outcome path ways (Ankley et al.).[15] We selected this latter model because of its applicability to observational studies and the sub stantial record of use in toxicology and ecology to support evidence based decisions related to risk assessment.[4,67,68] We populated our framework of adverse outcome pathway analysis, using the literature on mechanisms of antimicro bial resistance and assigned mechanistic evidence to a sequential pathway linking antimicrobial exposure of mi crobial communities to human exposure to drug resistant pathogens.

We focused on mechanisms that drive microbial response to antimicrobial stress through the emergence and dissemination of resistance as well as accumulation of resistance genes and organisms in reservoirs. To this model, we added evidence on the major pathways of human exposure to AMR pathogens from agricultural sources. The conditions of agricultural use facilitate many of the mech anisms in AMR emergence and transmission, such as hori zontal gene transmission and the frequency of multidrug resistant phenotypes. By including a further focus on agri cultural use, this assessment also supported the importance of the microbiome perspective. Moreover, it illustrated the role of agricultural use in expanding environmental re positories or resistomes through the direct contribution of agriculture to multiple pathways of release and from which AMR genes can be transferred to bacteria in human populations.

Conclusions

It is recognized that all uses of antimicrobials contribute to the emergence and dissemination of resistance.[69] In the context of increasing global threats of antimicrobial resistance, we need evidence to support effective interventions to control uses of antimicrobials in both health care and agriculture. The evi dence has been summarized in recent systematic reviews,[5,6,70] which reported associations observed between agricultural use of antimicrobials for all purposes and increased risks of AMR exposure of human populations. This article adds an analysis in support of the biological plausibility of these observations, using published methods based on a mechanistic approach. We conclude that this approach may be applicable to evaluate the evidence for biological plausibility as part of an overall assessment of evidence for action based systematic reviews on topics in which associations have been observed based on observational studies. This first application requires validation by application to other systematic reviews where the criterion of biological plausibility is of value.

Author statements

Acknowledgments

The authors thank their collaborating designer Lindsay Evans for her work in creating Fig. 5. This review started as a background article commissioned by the World Health Organization as part of the development of evidence sup porting guidelines for reducing the use of antimicrobials in food animal production. The WHO also provided support to E.K.S. for participating in the guidelines development group. J.D. was supported by the National Science Foun dation through a graduate research fellowship and the Center for a Livable Future through a CLF Lerner Fellow ship. They acknowledge the important comments and suggestions made by their colleagues in the WHO guide lines development group. The authors also acknowledge the contributions of the following colleagues: Kay Dickersin and Roberta Scherer of the US Cochrane Center, Daniele Mandrioli of the Ramazzini Institute, and their colleagues at Johns Hopkins Stefan Baral, Lori Rosman, and Alan Goldberg.

Ethical approval and consent to participate

Not applicable.

Funding

E.K.S. received funding from the World Health Organization to support her participation as a member of the Guidelines Development Group on antimicrobial use in food animal production; J.D. was supported by graduate fellowships from the Johns Hopkins University (Center for a Livable Future) and the National Science Foundation. L.R. received funding from Johns Hopkins University Hopkins Population Center (R24HD042854) and the National Eye Institute (Grant 1 UG1 EY020522), National Institutes of Health, Department of Health and Human Services, Bethesda, Md, USA. The sponsors had no role in the design of the study and collection, analysis, and interpretation of data in this article.

Competing interests

The authors declare that they have no competing interests.

Author contributions

E.K.S. and J.D. contributed equally to the conceptualization of this article and writing this manuscript. E.K.S. conducted literature searches and J.D. produced the original figures in the article. L.R. provided appropriate guidance on data collection and interpretation according to public health informationist standards and requirements. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and material

Data sharing not applicable to this article as no data sets were generated or analyzed during the present study.

REFERENCES

1. Hill AB. The environment and disease: association or causation? *Proc Roy Soc Med* 1965;**58**(1):295 300.
2. Baral SD, Wirtz A, Sifakis F, Johns B, Walker D, Beyrer C [Internet]. *The highest attainable standard of evidence (HASTE) for HIV/AIDS interventions: toward a public health approach to defining evidence*, vol. 127. Sage Publications, Inc.; 1974. p. 572 84. Public Health Reports [cited 2018 Jan 3].
3. Lewis SJ, Gardner M, Higgins JPT, Holly JMP, Gaunt TR, Perks CM, et al. Developing the WCRF International/University of Bristol methodology for identifying and carrying out systematic reviews of mechanisms of exposure cancer associations. *Cancer Epidemiol Biomark Prev* 2017:1667 76.
4. Becker RA, Dellarco V, Seed J, Kronenberg JM, Meek B, Foreman J, et al. Quantitative weight of evidence to assess confidence in potential modes of action. *Regul Toxicol Pharmacol* 2017 Jun 20;**86**:205 20 [Internet]. [cited 2017 May 5]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28232103.
5. Tang KL, Caffrey NP, Nóbrega DB, Cork SC, Ronksley PE, Barkema HW, et al. Restricting the use of antibiotics in food producing animals and its associations with antibiotic resistance in food producing animals and human beings: a systematic review and meta analysis. *Lancet Planet Heal* 2017;**1**(8):e316 27 [Internet]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2542519617301419.
6. Hoelzer K, Wong N, Thomas J, Talkington K, Jungman E, Coukell A. Antimicrobial drug use in food producing animals and associated human health risks: what, and how strong, is the evidence? *BMC Vet Res* 2017;**13**(1):211 [Internet]. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/s12917 017 1131 3.
7. Norris SL, Bero L. GRADE methods for guideline development: time to evolve? *Ann Intern Med* 2016;**165**(11):810 1.
8. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. *Am J Public Health* 2005;**95**.
9. USDA Chief Scientist Statement on WHO Guidelines on Antibiotics | USDA [Internet]. [cited 2017 Dec 16]. Available from: https://www.usda.gov/media/press releases/2017/11/07/usda chief scientist statement who guidelines antibiotics.
10. WHO. *WHO guidelines on use of medically important antimicrobials in food producing animals* [Internet]. WHO; 2017 [cited 2017 Dec 16]; Available from: http://who.int/foodsafety/publications/cia guidelines/en/.
11. Khan KS, Ball E, Fox CE, Meads C. Systematic reviews to evaluate causation: an overview of methods and application. *Evid Based Med* 2012;**17**(5):137 41 [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22491154%5Cnhttp://ebm.bmj.com/content/17/5/137.full.pdf.
12. Zaza S, Wright De Agüero LK, Briss PA, Truman BI, Hopkins DP, Hennessy MH, et al. Data collection instrument and procedure for systematic reviews in the guide to community preventive services. *Am J Prev Med* 2000;**18**(1 Suppl. 1):44 74.
13. Ertaylan G, Le Cornet C, van Roekel EH, Jung AY, Bours MJL, Damms Machado A, et al. A comparative study on the WCRF International/University of Bristol Methodology for systematic reviews of mechanisms underpinning exposure cancer associations. *Cancer Epidemiol Biomarkers Prev* 2017 [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28754794.
14. Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect* 2016;**124**:713 21.
15. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 2010;**29**(3):730 41.
16. Galbraith D, Levy PE, Sitch S, Huntingford C, Cox P, Williams M, et al. Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytol* 2010;**187**(3):647 65.
17. Leist M, Ghallab A, Graepel R, Marchan R, Hassan R, Bennekou SH, et al. Adverse outcome pathways: opportunities, limitations and open questions. *Arch Toxicol* 2017:1 29.
18. D'Agata EMC, Dupont Rouzeyrol M, Magal P, Olivier D, Ruan S. The impact of different antibiotic regimens on the emergence of antimicrobial resistant bacteria. *PLoS One* 2008;**3**(12):4 12.
19. Alban L, Ellis Iversen J, Andreasen M, Dahl J, Sønksen UW. Assessment of the risk to public health due to use of antimicrobials in pigs an example of pleuromutilins in Denmark. *Front Vet Sci* 2017;**4**(May):74 [Internet]. Available from: http://journal.frontiersin.org/article/10.3389/fvets.2017.00074/abstract.
20. Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, et al. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. *Environ Health Perspect* 2013;**121**(9):993 1001.
21. MacLean RC, Hall AR, Perron GG, Buckling A. The population genetics of antibiotic resistance: integrating molecular mechanisms and treatment contexts. *Nat Rev Genet* 2010;**11**(6):405 14 [Internet]. Available from: https://doi.org/10.1038/nrg2778.
22. Gillings MR. Evolutionary consequences of antibiotic use for the resistome, mobilome, and microbial pangenome. *Front Microbiol* 2013;**4**.
23. Graham JP, Nachman KE. Managing waste from confined animal feeding operations in the United States: the need for sanitary reform. *J Water Health* 2010;**8**(4):646 70.
24. Silbergeld EK, Davis M, Leibler JH, Peterson AE. One reservoir: redefining the community origins of antimicrobial resistant infections. *Med Clin* 2008;**92**(6):1391 407.

25. Perry JA, Wright GD. Forces shaping the antibiotic resistome. *BioEssays* 2014 Dec;**36**(12):1179 84 [Internet]. [cited 2017 May 5]. Available from: http://doi.wiley.com/10.1002/bies.201400128.
26. Silbergeld E. *Chickenizing farms and food*. Johns Hopkins Press; 2016.
27. Barbosa TM, Levy SB. The impact of antibiotic use on resistance development and persistence. *Drug Resist Updates* 2000;**3**(5):303 11 [Internet]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1368764600901675.
28. Arber W. Horizontal gene transfer among bacteria and its role in biological evolution. *Life (Basel, Switzerland)* 2014 May 16;**4**(2):217 24 [Internet]. [cited 2017 May 5]. Available from: http://www.mdpi.com/2075 1729/4/2/217/.
29. Hernando Amado S, Sanz García F, Blanco P, Martínez JL. Fitness costs associated with the acquisition of antibiotic resistance. *Essays Biochem* 2017;**61**:37 48 [Internet]. (October 2016). Available from: http://essays.biochemistry.org/content/ppebio/61/1/37.full.pdf.
30. Von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol* Feb 19, 2016:173 [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26925045.
31. Fleming A. *Nobel lecture: penicillin. Nobel lect physiol or med 1942 1962*. 1945. p. 83 93.
32. Hanahan D. Studies on transformation of Escherichia coli with plasmids. *J Mol Biol* 1983;**166**(4):557 80.
33. Yim G, Huimi Wang H, Davies FRSJ. Antibiotics as signalling molecules. *Philos Trans R Soc B Biol Sci* 2007;**362**(1483):1195 200 [Internet]. Available from: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2007.2044.
34. Overhage J, Bains M, Brazas MD, Hancock REW. Swarming of Pseudomonas aeruginosa is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *J Bacteriol* 2008;**190**(8):2671 9.
35. Boucher Y, Labbate M, Koenig JE, Stokes HW. Integrons: mobilizable platforms that promote genetic diversity in bacteria. *Trends Microbiol* 2007;**15**(7):301 9.
36. Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurence of antimicrobial resistance in fecal entercocci from food animals in Denmark. *Antimicrob Agents Chemother Agents Chemother* 2001;**45**(7):2054 9.
37. Cheng AC, Turnidge J, Collignon P, Looke D, Barton M, Gottlieb T. Control of fluoroquinolone resistance through successful regulation, Australia. *Emerg Infect Dis* 2012;**18**(9):1453 60.
38. ter Kuile BH, Kraupner N, Brul S. In: Heipieper H, editor. *The risk of low concentrations of antibiotics in agriculture for resistance in human health care*, vol. 363; 2016 Oct. fnw210 [cited 2017 May 5]. Available from: https://academic.oup.com/femsle/article lookup/doi/10.1093/femsle/fnw210.
39. Martinez JL. Antibiotics and antibiotic resistance genes in natural environments. *Science (80)* 2008 Jul 18;**321**(5887):365 7 [Internet]. [cited 2017 May 5]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18635792.
40. Cohen SP, McMurry LM, Hooper DC, Wolfson JS, Levy SB. Cross resistance to fluoroquinolones in multiple antibiotic resistant (Mar) Escherichia coli selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. *Antimicrob Agents Chemother* 1989 Aug;vol. 33(8):1318 25 [Internet]. [cited 2017 May 18]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2679373.
41. Cantón R, Morosini MI. Emergence and spread of antibiotic resistance following exposure to antibiotics. *FEMS Microbiol Rev* 2011;**35**(5):977 91.
42. Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, et al. Ceftriaxone resistant Salmonella infection acquired by a child from cattle. *N Engl J Med* 2000 Apr 27;**342**(17):1242 9 [Internet]. [cited 2017 May 5]. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200004273421703.
43. Argudín MA, Lauzat B, Kraushaar B, Alba P, Agerso Y, Cavaco L, et al. Heavy metal and disinfectant resistance genes among livestock associated methicillin resistant Staphylococcus aureus isolates. *Vet Microbiol* 2016 Aug 15;**191**:88 95 [Internet]. [cited 2017 May 5]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27374912.
44. Yu Z, Gunn L, Wall P, Fanning S. Antimicrobial resistance and its association with tolerance to heavy metals in agriculture production. *Food Microbiol* 2017;**64**:23 32.
45. You Y, Hilpert M, Ward MJ. Detection of a common and persistent tet(L) carrying plasmid in chicken waste impacted farm soil. *Appl Environ Microbiol* 2012 May;**78**(9):3203 13 [Internet]. [cited 2017 May 18]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22389375.
46. Cantón R, Ruiz Garbajosa P. Co resistance: an opportunity for the bacteria and resistance genes. *Curr Opin Pharmacol* 2011 Oct;**11**(5):477 85 [Internet]. [cited 2017 May 5]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1471489211001299.
47. Graham DW, Knapp CW, Christensen BT, McCluskey S, Dolfing J. Appearance of β lactam resistance genes in agricultural soils and clinical isolates over the 20th century. *Sci Rep* 2016 Feb 16;**6**(1):21550 [Internet]. [cited 2017 May 18]. Available from: http://www.nature.com/articles/srep21550.
48. Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 2010;**74**(3):417 33 [Internet]. Available from: http://mmbr.asm.org/cgi/content/abstract/74/3/417.
49. Shigemura K, Tanaka K, Adachi M, Yamashita M, Arakawa S, Fujisawa M. Chronological change of antibiotic use and antibiotic resistance in Escherichia coli causing urinary tract infections. *J Infect Chemother* 2011;**17**(5):646 51 [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21487942.
50. Molton JS, Tambyah PA, Ang BSP, Ling ML, Fisher DA. The global spread of healthcare associated multidrug resistant bacteria: a perspective from Asia. Weinstein RA, editor *Clin Infect Dis* 2013 May 1;vol. 56(9):1310 8 [Internet]. [cited 2017 May 5]. Available from: https://academic.oup.com/cid/article lookup/doi/10.1093/cid/cit020.
51. Skurnik D, Clermont O, Guillard T, Launay A, Danilchanka O, Pons S, et al. Emergence of antimicrobial resistant *Escherichia coli* of animal origin spreading in humans. *Mol Biol Evol* 2016 Apr;**33**(4):898 914 [Internet]. [cited 2017 May 5]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26613786.
52. Mollenkopf DF, Stull JW, Mathys DA, Bowman AS, Feicht SM, Grooters SV, et al. Carbapenemase Producing enterobacteriaceae recovered from the environment of a swine farrow to finish operation in the United States. *Antimicrob Agents Chemother* 2017 Feb 5;**61**(2) [Internet]. [cited 2017 May 5]. AAC.01298 16. Available from: http://aac.asm.org/lookup/doi/10.1128/AAC.01298 16.
53. Ravi A, Avershina E, Foley SL, Ludvigsen J, Storrø O, Øien T, et al. *The commensal infant gut meta mobilome as a potential reservoir for persistent multidrug resistance integrons*, vol. 5; 2015 Oct 28. p. 15317 [cited 2017 May 5]. Available from: http://www.nature.com/articles/srep15317.
54. Muurinen J, Stedtfeld R, Karkman A, Pärnänen K, Tiedje J, Virta M. Influence of manure application on the environmental resistome under Finnish agricultural practice

with restricted antibiotic use. *Environ Sci Technol* 2017;**51**(11):5989 99.
55. Cleary DW, Bishop AH, Zhang L, Topp E, Wellington EMH, Gaze WH. Long term antibiotic exposure in soil is associated with changes in microbial community structure and prevalence of class 1 integrons. *FEMS Microbiol Ecol* 2016;**92**(10).
56. Davis MF, Price LB, Liu CM HH, Silbergeld EK. An ecological perspective on U.S. industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug resistant bacteria from agricultural environments. *Current Opinion Microbiol* Jun, 2011:244 50 [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21621451.
57. Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, et al. Antimicrobial use in aquaculture re examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol* 2013;**15**(7):1917 42.
58. Zhu YG, Zhao Y, Li B, Huang CL, Zhang SY, Yu S, et al. Continental scale pollution of estuaries with antibiotic resistance genes. *Nat Microbiol* 2017;**2**.
59. You Y, Silbergeld EK. Learning from agriculture: understanding low dose antimicrobials as drivers of resistome expansion. *Front Microbiol* 2014;**5**.
60. Hayes JR, English LL, Carr LE, Wagner DD, Joseph SW. Multiple antibiotic resistance of Enterococcus spp. isolated from comemercial poultry production environments. *Apllied Environ Microbiol* 2004;**70**(10):6005 11.
61. Berriman ADC, Clancy D, Clough HE, Armstrong D, Christley RM. Effectiveness of simulated interventions in reducing the estimated prevalence of Salmonella in UK pig herds. *PLoS One* 2013;**8**(6).
62. van Hoek AHAM, Veenman C, van Overbeek WM, Lynch G, de Roda Husman AM, Blaak H. Prevalence and characterization of ESBL and AmpC producing Enterobacteriaceae on retail vegetables. *Int J Food Microbiol* 2015;**204**:1 8 [Internet]. [cited 2017 May 18]. Available from: http://www.sciencedirect.com/science/article/pii/S0168160515001531.
63. Ben Said L, Jouini A, Klibi N, Dziri R, Alonso CA, Boudabous A, et al. Detection of extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. *Int J Food Microbiol* 2015 Jun 16;**203**:86 92 [Internet]. [cited 2017 May 18]. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168160515001129.
64. Leibler JH, Dalton K, Pekosz A, Gray GC, Silbergeld EK. Epizootics in industrial livestock production: preventable gaps in biosecurity and biocontainment. *Zoonoses Public Heal* 2017;**64**:137 45.
65. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* 2012;**3**(1):1 6.
66. Agersø Y, Wulff G, Vaclavik E, Halling Sørensen B, Jensen LB. Effect of tetracycline residues in pig manure slurry on tetracycline resistant bacteria and resistance gene tet(M) in soil microcosms. *Environ Int* 2006;**32**(7):876 82.
67. Villeneuve DL, Crump D, Garcia Reyero N, Hecker M, Hutchinson TH, LaLone CA, et al. Adverse outcome pathway (AOP) development I: strategies and principles. *Toxicol Sci* 2014;**142**(2):312 20.
68. Patlewicz G, Simon TW, Rowlands JC, Budinsky RA, Becker RA. Proposing a scientific confidence framework to help support the application of adverse outcome pathways for regulatory purposes. *Regul Toxicol Pharmacol* 2015;**71**(3):463 77 [Internet]. Available from: https://doi.org/10.1016/j.yrtph.2015.02.011.
69. O'Brien TF. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. *Clin Infect Dis* 1997 Jan;**24**(Suppl. 1):S2 8 [Internet]. [cited 2017 May 18]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8994775.
70. Hoelzer K, Wong N, Thomas J, Talkington K, Jungman E, Coukell A. Antimicrobial drug use in food producing animals and associated human health risks: what, and how strong, is the evidence? *BMC Vet Res* 2017;**13**(1):211 [Internet]. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/s12917 017 1131 3.